AUROVERTIN-SENSITIVE PHOSPHATE ACTIVATION OF MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE

Peter MITCHELL and Jennifer MOYLE

Glynn Research Laboratories, Bodmin, Cornwall, England

Received 17 July 1970

1. Introduction

The rate of hydrolysis of ATP by the coupling factor 1 (F_1) component of the ATPase of beef heart mitochondria is not apparently changed by 40 mM phosphate [1], and a low (0.24 mM) phosphate concentration has little effect on the complete (oligomycin-sensitive) ATPase of rat liver mitochondria [2]. In recent unpublished work we observed that ATP hydrolysis in intact rat liver mitochondria showed an auto-catalytic time-course when exit of phosphate was inhibited by *N*-ethylmaleimide; and we have therefore investigated the effect on the ATPase of phosphate concentrations comparable to those in normal mitochondria.

The observations described here on the kinetics of the ATPase of a sonic preparation from rat liver mitochondria show that 20 mM inorganic phosphate induces a seven-fold increase in V_{max} and time-dependent changes in $(K_i)_{ADP}$, but little or no change in $(K_m)_{ATP}$. The low V_{max} of the ATPase in presence of oligomycin is likewise increased seven-fold by 20 mM phosphate; but the high V_{max} of the phosphate-activated ATPase is depressed to the phosphate-free level by aurovertin.

2. Materials and methods

Rat liver mitochondria were isolated as described previously [3]. Sonic particles were prepared [2] from mitochondrial suspensions and were stored at 4° in 250 mM sucrose. As before [2], the ATPase activity was routinely measured at 25° in 3.3 ml of medium near pH 7 containing 150 mM KCl, 3.3 mM glycylglycine, 5 mM $MgCl_2$ and sonic particles corresponding to 1.6 mg of protein, using the pH method of Nishimura, Ito and Chance [4] in presence of 1 μM carbonylcyanide *p*-trifluoromethoxy phenylhydrazone (FCCP) to avoid possible effects of proton translocation. The ATP was added as a pulse (20 μl) of 150 mM KCl containing equimolar ATP and $MgCl_2$ adjusted to pH 7.05. The ATPase activity (with or without addition of 20 mM phosphate) was unchanged by 5 μM atractyloside or after incubation for 20 min with 1 mM *N*-ethylmaleimide, confirming that translocation of phosphate, ATP or ADP was not rate-limiting. Decreasing the 5 mM $MgCl_2$ of the routine ATPase assay medium to 0.2 mM did not affect the ATPase activity in presence or absence of 2 mM P_i.

3. Results and discussion

Fig. 1a shows Lineweaver-Burk plots of ATPase activity over a range of P_i concentration. Phosphate activates non-competitively with respect to ATP, $(K_m)_{ATP}$ being 106 μM in presence or absence of phosphate. Fig. 1b shows that V_{max} approaches a limiting value above 20 mM P_i, the half-maximal activation being near 5 mM P_i. Considerable variation of the V_{max} values was observed from preparation to preparation, and mean values from six separate sonic particle preparations have therefore been plotted in fig. 1b with standard deviations represented by the vertical lines.

Fig. 2 shows the effects of 20 mM P_i and of aurovertin (12.5 mg/g of particle protein) on the time-course of ATP hydrolysis. The activating effect of phosphate was abolished by the presence of aurovertin. Thus, although, as shown previously [2], the ATPase

North-Holland Publishing Company – Amsterdam

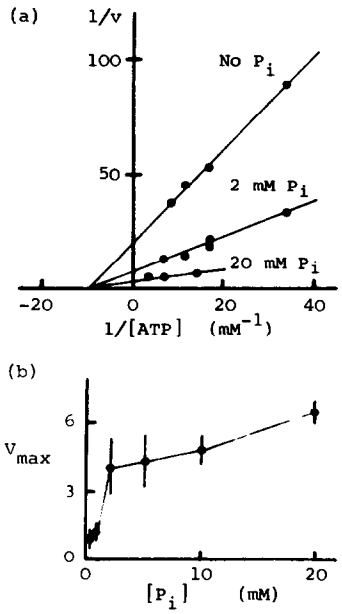

Fig. 1. Activation of sonic particle ATPase by inorganic phosphate. Sonic particles (1.6 mg of protein) were suspended in 3.3 ml of a medium containing 150 mM KCl, 3.3 mM glycylglycine, 5 mM $MgCl_2$ and 1 μM FCCP at pH 7.0–7.1 at 25°. P_i was added to the routine medium as indicated, and ATPase activity initiated by pulses of ATP.

a) Lineweaver-Burk plots of the relationship between initial ATP concentration, P_i concentration, and initial rate of ATP hydrolysis;

b) relationship between V_{max} and [P_i]. Values of V_{max} are mean values from six separate sonic particle preparations, and standard deviations are indicated by the vertical lines. V_{max} in absence of P_i is given a value of unity, and corresponds to the hydrolysis of 3.4 μmoles of ATP/sec g of particle protein.

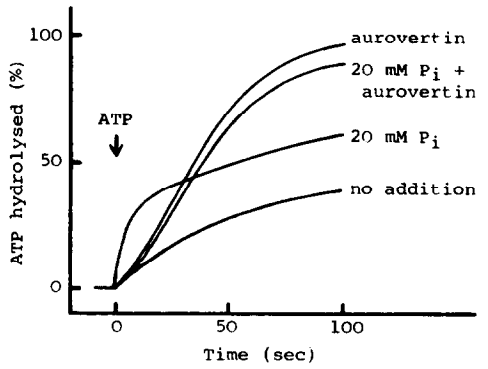

Fig. 2. Effect of P_i and aurovertin on the time-course of ATP hydrolysis by sonic particles. Conditions were as in the experiments of fig. 1. In addition, where indicated, 20 mM P_i and aurovertin (12.5 mg/g of particle protein) were present. At the arrow, pulses of 0.5 μmoles of ATP were added.

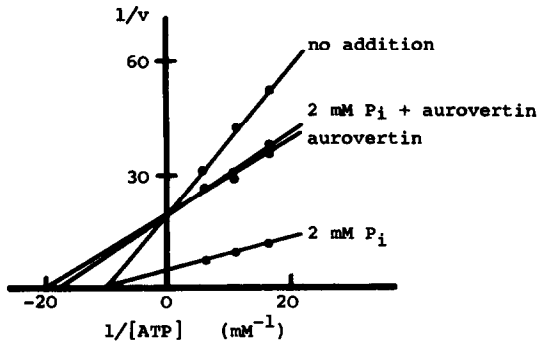

Fig. 3. Lineweaver-Burk plots of the relationship between initial ATP concentration, in presence or absence of 2 mM P_i and aurovertin (12.5 mg/g of particle protein), and the initial rate of ATP hydrolysis by sonic particles. Conditions were as in fig. 1.

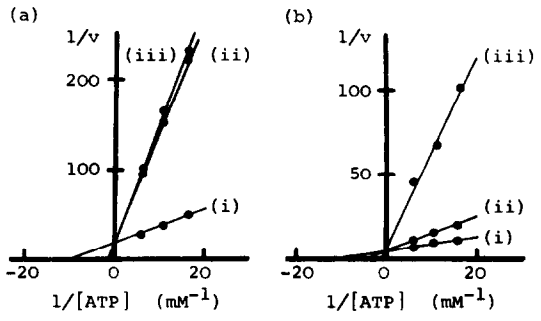

Fig. 4. Lineweaver-Burk plots of the relationship between initial ATP concentration and the initial rate of ATP hydrolysis by sonic particles, with no ADP (i), in presence of 60 μM ADP added simultaneously with the ATP pulse (ii), and with 60 μM ADP added 60 sec before the ATP pulse (iii): a, in absence of P_i; b, in presence of 2 mM P_i. Conditions were as in fig. 1.

activity represented by V_{max} or by the initial velocity was not depressed by aurovertin in the absence of phosphate, when 20 mM phosphate was present, aurovertin depressed the initial velocity of the phosphate-activated ATPase about seven-fold (i.e. to the normal phosphate-free rate). Similar results were obtained using 2 mM P_i.

The effects of 2 mM P_i and of aurovertin separately and together on V_{max} and $(K_m)_{ATP}$ extrapolated to zero time after ATP addition are shown in the Lineweaver-Burk plots of fig. 3. These observations, and similar observations using 20 mM P_i, show that P_i does not affect the initial value of $(K_m)_{ATP}$ either in the presence or in the absence of aurovertin.

Using the rationale described previously [2], the time-course of ATP hydrolysis in presence of aurovertin (fig. 2) indicates that the same time-dependent change of $(K_m)_{ATP}$ occurs in the presence of P_i as previously observed in its absence [2]. The time-course of ATP hydrolysis in presence of 20 mM P_i but in absence of aurovertin (fig. 2) likewise indicates that there is a time-dependent change of one or more of the coefficients: $(K_m)_{ATP}$, $(K_i)_{ADP}$, V_{max}. Changes of the individual coefficients were studied by adding ADP either simultaneously with the ATP pulse or 60 sec in advance. The Lineweaver-Burk plots of fig. 4 show that: (a) in absence of phosphate, $(K_i)_{ADP}$ was not time-dependent; and (b) in presence of 2 mM phosphate, $(K_i)_{ADP}$ was initially 50 μM, compared with 9 μM in absence of P_i, and changed to 4.5 μM at 60 sec after ADP addition. The same initial and 60 sec values for $(K_i)_{ADP}$ were obtained in presence of 20 mM P_i. The time-course of the phosphate-activated ATPase shown in fig. 2 may be accounted for by an initial phosphate-activated value of 50 μM for $(K_i)_{ADP}$ which falls to 4.5 μM during the first 20 sec, while $(K_m)_{ATP}$ remains constant at 106 μM and V_{max} is also constant (the value depending on $[P_i]$) throughout ATP hydrolysis.

The V_{max} of the ATPase after preincubation for 15 min with 6.8 mg of oligomycin/g of particle protein (which is 7% of the total V_{max} whereas $(K_m)_{ATP}$ and $(K_i)_{ADP}$ are the same as for the normal ATPase [2]) was increased by factors of 3.6 and 6.9 when 2 mM and 20 mM P_i was present respectively. In the absence of oligomycin, 2 mM and 20 mM P_i increased V_{max} by factors of 3.5 and 6.7 in the same preparation. The results were not significantly affected when the preincubation with the oligomycin preceded the addition of and activation by the phosphate.

Using essentially the same technique as for the sonic particles we have observed that at 25°, in an anaerobic medium containing 150 mM KCl, 5 mM sucrose, 3.3 mM glycylglycine, 1 mM potassium ethylenediamine tetracetate and 1 μM FCCP near pH 7, the initial rate of hydrolysis of ATP (initial concentration 150 μM) by intact rat liver mitochondria (1 mg protein/ml) was 1.6 and 2.1 times faster when 2 mM and 20 mM inorganic phosphate respectively was also present. Similar activations of the ATPase of intact mitochondria by 2 mM and 20 mM P_i were observed when the 1 μM FCCP of the above medium was replaced by valinomycin (25 μg/g of protein) and ATPase activity was accompanied by proton translocation. Thus, in spite of the likelihood of partial suppression of ATPase activation by the rate-limiting effect of the ATP/ADP antiporter [5], the proton-translocating ATPase system of intact mitochondria can be seen to be activated by phosphate, and this property of the ATPase of the sonic particles is not, therefore, a preparative artefact.

The biochemical mechanism of the activation of the proton-translocating ATPase of rat liver mitochondria by inorganic phosphate (an end-product of the reaction) is not self-evident; but the fact that phosphate-activation is abolished by aurovertin (which reacts with F_1 – presumably the ATP, ADP and P_i accepting component), but not by oligomycin (which reacts with another component) [6], may possibly have some bearing on the site of action of phosphate on the ATPase system. The potent activating effect of phosphate appears to have escaped detection until now, and we draw attention to it in this brief paper in view of its obvious relevance to the behaviour of the reversible ATPase involved in oxidative phosphorylation. The stimulation by P_i of ADP-ATP exchange observed by Groot [7] may, for example, be explained by the present observations.

In an extension of the present work (to be published in detail elsewhere) we have observed an activating effect by arsenate, quantitatively very similar to that by phosphate. These observations reveal an important additional factor that must be considered in studies of the stimulating effect of arsenate on the ATPase of mitochondria [8–11].

References

[1] M.E.Pullman, H.S.Penefsky, A.Datta and E.Racker, J. Biol. Chem. 235 (1960) 3322.
[2] P.Mitchell and J.Moyle, FEBS Letters 6 (1970) 309.
[3] P.Mitchell and J.Moyle, Biochem. J. 104 (1967) 588.
[4] M.Nishimura, T.Ito and B.Chance, Biochim. Biophys. Acta 59 (1962) 177.
[5] E.Duée and P.V.Vignais, J. Biol. Chem. 244 (1969) 3920.
[6] H.A.Lardy and S.M.Ferguson, Ann. Rev. Biochem. 38 (1969) 991.
[7] G.S.P.Groot, Biochim. Biophys. Acta 180 (1969) 439.
[8] G.F.Azzone and L.Ernster, J. Biol. Chem. 236 (1961) 1510.
[9] J.B.Chappell and A.R.Crofts, Biochem. J. 95 (1965) 707.
[10] H.F.Ter Welle and E.C.Slater, Biochim. Biophys. Acta 143 (1967) 1.
[11] L.Ernster, C.P.Lee and S.Janda, in: Biochemistry of Mitochondria, eds. E.C.Slater, Z.Kaniuga and L.Wojtczak (Academic Press, London, 1967) p. 29.